Novel Functional Polysaccharides as Edible Coatings for Cheese

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***Abstract*.**

One of the problems occurring during cheese ripening and later on, throughout the distribution chain, is the occurrence of molds and the loss of water. Usually, this problem is solved by the use of synthetic coatings where an antimicrobial agent is introduced. Polysaccharide coatings have an oil-free appearance, a low caloric content and can be used to increase the shelf life of foods. The objective of this work was to study the ability of polysaccharides from different and novel sources to be used as coatings for cheese. The tested materials were: chitosan, galactomannan of Gleditsia triacanthos *and agar of* Glacilariae birdiae*. Different formulations were tested with the addition of plasticizer (glycerol and sorbitol), oil and Tween 80. The surface properties of the cheese and the wetting capacity of the coatings on the cheese (in terms of the spreading coefficient) were determined. Based on the values of the spreading coefficient of the polysaccharides solutions the three best solutions for each polysaccharide were chosen. For the chosen solutions, the water, oxygen and carbon dioxide permeability of the films were determined, as well as solubility, opacity and chromaticity. The solutions of* G. triacanthos *present the best properties to coat the cheese. The O_2 consumption and CO_2 production rate, as well as the weight loss, of the cheese with and without coating were measured. The gas exchange rates of the cheese were found to decrease in the coated cheese. Also the weight loss from the cheese was lower in the coated cheese.*

Keywords. Edible coatings, Edible films, Polysaccharides, Functional, Cheese.

Introduction

In the last years, the food and packaging industries joined efforts to reduce the amount of food packaging materials, once the environmental issues became important to the consumer. As an answer to that concern several problems were addressed in order to the commercial use of biobased primary food packaging materials. These problems include degradation rates under various conditions, changes in mechanical properties during storage, potential for microbial growth, and release of harmful compounds into the packaged food product (Karina, Per, Grete, Mark, Mette, Nils & Grith, 1999).

The future generation of packaging materials will be derived from renewable resources. These materials will ideally be biodegradable. However, natural polymeric materials vary in their rate of degradation in the environment, and some proteins, for example, cannot presently be classified as degradable because of standard definitions (Cooke, 1990)**.** The edible films can improve shelf life and food quality with good and selective barriers to moisture transfer, oxygen uptake, lipid oxidation, losses of volatiles aromas and flavours (Kester & Fennema, 1986), better visual aspect, and reduction of the microbiologic contamination (Nisperos-Carriedo, 1994).

Cheese is a complex food product consisting mainly of casein, fat and water. Several researchers have recommended that fresh cheeses (e.g. cream cheese, decorated cream cheese, soft cheese, and cottage cheese) are packaged in modified atmospheres with N_2 and/or CO_2 replacing the O_2 in the package (Mannheim *&* Soffer, 1996; Fedio, Macleod & Ozimek, 1994). However spoilage caused by yeast and especially bacteria may still occur even at very low O_2 and elevated CO_2 levels (Westall *&* Filtenborg, 1998). Semi-soft and hard cheeses (whole, sliced or shredded) have a relatively high respiration rate, which require a packaging material somewhat permeable to CO_2 to avoid blowing of the packaging. Meanwhile, oxygen must be kept out to avoid fungal spoilage and oxidation of the cheese. The primary spoilage organism on these cheeses is *Penicillium commune* (Lund, Filtenborg & Frisvad, 1995). Mould ripened cheeses, such as white cheeses (Brie/Camembert) and blue-veined cheeses (Danablue and Roquefort), contain active fungal cultures. As a consequence, the oxygen content should not be too low as this may cause anaerobic respiration and production of flavours. Additionally, a change in atmospheric composition can cause a change in the microbiota. Instead these products require a balanced oxygen and carbon dioxide atmosphere to prolong shelf-life (Nielsen & Haasum, 1997; Haasum & Nielsen, 1998).

The most important factors that affect cheese stability are a_w and pH. Water activity depends mainly on moisture and salt contents. During ripening, a_w is not constant but decreases until the cheese surface is in equilibrium with the surrounding atmosphere. During cheese manufacture, the pH due to the effect of the fermentation of lactose to lactic acid until a level that inhibits the growth of many pathogenic bacteria (Robertson, 1993). While the packaging does not have influence on the pH of the cheese, the water vapour transmission rate through the packaging material is crucial for controlling the a_w. Additional environmental factors which must be considered in selecting a material for cheese coating are light and oxygen. Light promotes fat oxidation, which in turn is responsible of off-flavour. The oxygen in contact with the cheese contributes to the oxidation of fats and to the growth of undesirable microorganisms (Robertson, 1993). All these factors affect not only cheese's physical characteristics but also its flavour during storage. In fact many different compounds contribute to cheese flavour and most of them form during cheese ripening. The breakdown of milk proteins, fats, lactose and citrate during ripening gives rise to a series of volatile and non volatile compounds which may be related to total flavour. Sensory experiments confirm the contribution given by fat-derived compounds to cheese flavour (Arora, Cormier, & Lee, 1995; Buchin, Delage, Duboz, B erdague, Beuvier, & Pochet, 1998; Kubickova & Grosch, 1998).

Materials and Methods

Materials

Edible coatings solutions were prepared with: chitosan with a degree of deacetylation of 90 % approximately (Aqua Premier Co., Thailand), galactomannan extracted from *G. triacanthos* seeds, agar extracted from *G. birdiae* seaweed; corn oil (Sovena, Portugal), glycerol 87% (Panreac, Spain) and sorbitol 97% (Acros Organics, Belgium) as plasticizers, Tween 80 (Acros Organics, Belgium) as surfactant, lactic acid (Merck, Germany) and distilled water. A commercial processed cheese was obtained from A Queijo Saloio S.A without any coating, being the samples stored at 5 ºC until further use.

Polysaccharide extraction

Galactomannan (*G. triacanthos*)

The polysaccharide extraction was performed with ethanol and distilled water. In this process the seeds are removed from the pods, cleaned and put in a blender. The endosperm is suspended in ethanol at 70 ºC during 15 minutes. The ethanol is decanted and distilled water is then added in the proportion of 1:100. This mixture is left during approximately 1 hour and then mixed in a blender during 5 min. The purification of the galactomannan is achieved by filtering it through nylon, followed by centrifugation at 9 000 G (Sigma 4K15, Germany) during 10 minutes. The precipitation of the galactomannan was achieved by adding the supernatant to pure ethanol in the proportion 1:2. The precipitated galactomannan is lyophilized and kept in a dry place until further use.

Agar (*G. birdae*)

The polysaccharide extraction was performed with ethanol and distilled water. In this process the seaweed was cleaned, dried and milled. It was mix in water (1.5 % w/v) during 15 h at 25 ºC. Then was a centrifugation at 10 000 G (Sigma 4K15, Germany) during 10 minutes. The precipitation of the polysaccharides was achieved by adding the supernatant to pure ethanol in the proportion 1:3. The polysaccharides is lyophilized and kept in a dry place until further use.

Preparation of chitosan (C) solution and film

The coating solutions were prepared dissolving the chitosan (0.5 or 1.5 % w/v) in a 1% (v/v) lactic acid solution with agitation using a magnetic stirrer during 2 hours at room temperature (20 ^{o}C); Tween 80 was also added as a surfactant at concentrations of 0.2 % (w/v). Corn oil was added in concentrations of 0.5 % (w/v), with agitation during 20 minutes at 60 ºC. As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added in concentrations between 0.5 and 2.0 % (w/v). A constant amount (13 mL) chitosan solution was cast onto a 5.7 cm diameter glass plate in order to maintain the film thickness. The films were dried in an oven at 35 °C. These solutions correspond to samples 1 to 16, in Table 2, for chitosan.

Preparation of *G. triacanthos* (GT) solution and film

The coating solutions were prepared dissolving the galactomannan of *G. triacanthos* (GT) (0.5 or 1.5 % w/v) in distilled water with agitation using a magnetic stirrer during 24 hours at room temperature (20 ^{o}C). As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added in concentrations between 0.5 – 2.0 % (w/v). Corn oil was added in concentrations of 0.5 % (w/v), with agitation during 20 min at 60 ºC. A constant amount (13 mL) of GT solution was cast onto a 5.7 cm diameter glass plate in order to maintain the film thickness. The films were dried in an oven at 35 °C. These solutions correspond to samples 1 to 16, in Table 2, for *G. triacanthos.*

Preparation of *G. birdiae* (GB) solution and film

The coating solutions were prepared dissolving the agar of *G. birdiae* (GB) (0.5 or 1.5 % w/v) in distilled water with agitation using a magnetic stirrer during 20 minutes at 60 ºC. As plasticizers, glycerol and a mixture of glycerol/sorbitol (50:50) were added in concentrations between 0.5 and 2.0 % (w/v). Corn oil was added in a concentration of 0.5 % (w/v). A constant amount (13 mL) of solution was cast onto a 5.7 cm diameter glass plate in order to maintain the film thickness. The films were dried in an oven at 35 °C. These solutions correspond to samples 1 to 16, in Table 2, for *G. birdiae.*

Film thickness

The film thickness was measured with a digital micrometer (Mitutoyo, Japan). Five thickness measurements were taken on each testing sample in different points and the mean values were used in water vapour permeability (WVP), oxygen permeability (O_2P) and dioxide carbon permeability (CO_2P).

Wettability

In order to know the wetting properties of the polysaccharide solutions over cheese, contact angle (θ) and surface tension (γ_L) were determined by a contact angle meter (OCA 20, Dataphysics, Germany). The surface tension of the coating solution was measured by the pendent drop method and Laplace-Young approximation (Song & Springer. 1996). The contact angle between the solution and the cheese surface was measured by the sessile drop method. The estimation of the critical surface tension (γ_c) of the cheese surface was obtained by extrapolation from the Zisman plot (Zisman, 1964), which was built using water, formamide and bromonaphthalene (Merck, Germany) as reference liquids. Twenty replicates of contact angle and surface tension measurements were obtained at 21.3 ± 0.2 ºC.

Water vapor permeability measurement

The measurement of water vapor permeability (WVP) was determined gravimetrically based on ASTM E96-92 method (McHugh, Avena-Bustillos & Krochta, 1993; Guillard, Broyart, Bonazzi, Guilbert & Gontard, 2003). The film was sealed on the top of a permeation cell containing distilled water (100 % RH; 2337 Pa vapor pressure at 20 °C), placed in a desiccator at 20 °C and 0 % RH (0 Pa water vapour pressure) with silica. The cups were weighed at intervals of 2 hours during 10 hours. Steady-state and uniform water pressure conditions were assumed by keeping the air circulation constant outside the test cup by using a miniature fan inside the desiccator (McHugh, Avena-Bustillos & Krochta, 1993). The slope of weight loss versus time was obtained by linear regression. The measurements were repeated three times to each film.

Oxygen permeability (O_2P)

Oxygen permeability (O_2P) was determined based on the ASTM (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber O_2 is supplied at a controlled flow rate to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at a controlled flow. This nitrogen acted as a carrier for the O_2 and the flow leaving this chamber was connected to an O_2 sensor. The flows of the two chambers were connected to a manometer to ensure the equality of pressures between both compartments. As the O_2 was carried continuously by nitrogen flow, it was considered that O_2 partial pressure in the upper compartment is null, therefore ΔP is equal a 1 atm. The measurements were repeated three times to each film.

Carbon dioxide permeability (CO_2P)

Carbon dioxide permeability (CO_2P) was determined based on the ASTM (2002) method. The films were sealed between two chambers, having each one two channels. In the lower chamber CO_2 is supplied at a controlled flow rate to keep its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at a controlled flow. This nitrogen acted as a carrier for the CO_2 and the flow leaving this chamber was collected for CO_2 quantification. The flows of the two chambers were connected to a manometer to ensure the equality of pressures between both compartments. As the CO_2 was carried continuously by nitrogen flow, it was considered that CO_2 partial pressure in the upper compartment is null, therefore ΔP is equal to 1 atm. To determine CO_2 concentration 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 ºC with a column Porapak Q 80/ 100 mesh 2 m x 1/8" x 2 mm SS, using a flame ionization detector (FID) at 110 ºC. Helium at 23 mL/min was used as carrier gas. A standard mixture containing 10 % CO_2, 20 % O_2 and 70 % N_2 was used for calibration. The measurements were repeated three times to each film.

Solubility

The film solubility in water was determined according to the method reported by Gontard, Duchez, Cuq, & Guilberts (1994). It was defined by the content of dry matter solubilized after 24 h immersion in water. The initial dry matter content of each film was determined by drying to constant weight in an oven at 105 ºC. Two disks of film (2 cm diameter) were cut, weighed, and immersed in 50 mL of water. After 24 h of immersion at 20 ºC with occasional agitation, the pieces of films were taken out and dried to constant weight in an oven at 105 ºC, to determine the weight of dry matter which was not solubilized in water.

Color and opacity

The color of films was determined with a Minolta colorimeter (CR 300; Minolta, Japan). A white standard color plate (Y=93.5, x=0.3114, y=0.3190) for the instrument's calibration was used as a background for color measurements of the coated films, and the CIE L^* a^* b^* values of each films were evaluated by reflectance measurement. The opacity of the samples was determined according to Hunter lab method, as the relationship between the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w).

O_2 and CO_2 transfer rates

The O_2 and CO_2 transfer rates in cheese were measured by placing one cheese inside an hermetic jar and closing it. The air circulation was promoted inside the jar by using a miniature fan. The atmosphere inside the jar was measured by drawing the gas samples with a 1 mL syringe through a septum fitted in the jar lid. The O_2 and CO_2 content in the jar was determined using a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110 ºC with a column mol.sieve 5A 80/ 100 mesh 1 m x 1/8" x 2 mm to separate the O_2 and a column Porapak Q 80/ 100 mesh 2 m x 1/8 "x 2 mm SS to separate the CO_2 using a flame ionization detector (FID) at 110 ºC. Helium at 23 mL/min was used as carrier gas. A standard mixture containing 10 % CO_2, 20 % O_2 and 70 % N_2 was used as standard for calibration.

Weight loss and relative humidity

The weight loss and relative humidity were measured. The cheese was weighed at the beginning (IW) of the experiment and at the end (FW), being the results expressed as the relative weight loss (RWL), defined as:

$$RWL = \frac{IW - FW}{IW} \times 100 \qquad \textbf{Equation 1}$$

The change in relative humidity (RH) inside jar atmosphere was followed using a sensor (hygrometer HD 8501 H) fitted inside the jar.

Statistical analysis

Statistical analyses were performed using Analysis of Variance (ANOVA) and linear regression analysis. The Tukey test (α = 0.05) was used to determine any significance of differences between specific means (SigmaStat 3.1, 2004, Excel, 2003, USA)

Results and Discussion

Critical Surface Tension and Surface Tension of cheese

The surface tension and critical surface tension of the cheese allows the characterization of the surface skin of the cheese. According to Zisman (1964), in systems having a surface tension lower than 100 mN/m (low energy surfaces), the contact angle formed by a drop of liquid on a solid surface will be a linear function of the surface tension of the liquid, γ_{LV}, (where phase *V* is air saturated with the vapour of liquid, *L*). That determination allows the application of the method to determinate the wettability.

In Table 1 is presented the values of the critical surface tension and surface tension of the cheese. Cheese surface is a low-energy surface (< 100 mN), and present a higher dispersive component, which shows the ability of the cheese surface to participate in dispersive (non-polar) interactions, considered to be partly hydrophobic (Van Oss, 1995).

Table 1 – Critical Surface tension and surface tension of the analysed cheese

Critical Surface Tension (mN/m)	Surface tension (mN/m)	Polar component (mN/m)	Dispersive component (mN/m)
18.33 ± 0.10	37.79 ± 0.76	7.87 ± 0.37	29.93 ± 0.41

(data represent the mean ± standard deviation, 95 % e *n* = 60, measured at T = 21.3 ± 0.2 ºC)

Wettability

The wettability was studied by determining the values of the spreading coefficient (*Ws*) and the works of adhesion (*Wa*) and cohesion (*Wc*). The adhesive forces promote the liquid spreading in a solid surface and the cohesive forces promote its contraction. The wetting behaviour of the solutions will mainly depend on the balance between these forces.

The results show (Table 2) that, depending on the source of polysaccharide, the values of *Ws* are statistically different. Using the various solutions, the best (higher) value of *Ws* was determined for cheese surface (Tukey test, $p < 0.05$). The best values are filled in gray.

Table 2 – Spreading coefficient (*Ws*) obtained for solutions in the cheese.

Sample	Poly. (w/v)	Glycerol (w/v)	Glycerol/ Sorbitol (w/v)	Oil (w/v)	Chitosan	*G. triacanthos*	*G. birdiae*
1	**0.5**	**0.5**	**-**	**-**	-28.97 ± 6.23	-42.94 ± 5.49	-45.85 ± 5.83
2	**0.5**	**2.0**	**-**	**-**	-29.81 ± 3.79	-57.84 ± 7.89	-36.49 ± 3.89
3	**0.5**	**0.5**	**-**	**0.5**	-34.50 ± 3.07	-37.05 ± 4.41	-55.46 ± 4.83
4	**0.5**	**2.0**	**-**	**0.5**	-35.76 ± 5.48	-41.69 ± 4.34	-47.37 ± 3.69
5	**0.5**	**-**	**0.5**	**-**	-34.46 ± 4.31	-49.69 ± 6.02	-49.62 ± 3.86
6	**0.5**	**-**	**2.0**	**-**	-29.96 ± 3.99	-54.79 ± 4.27	-45.69 ± 4.45
7	**0.5**	**-**	**0.5**	**0.5**	-36.62 ± 3.92	-51.01 ± 4.40	-52.81± 4.33
8	**0.5**	**-**	**2.0**	**0.5**	-36.49 ± 4.70	-41.93± 3.99	-47.97 ± 5.32
9	**1.5**	**0.5**	**-**	**-**	-38.31 ± 3.69	-58.97 ± 5.37	-39.24 ± 4.45
10	**1.5**	**2.0**	**-**	**-**	-38.95 ± 3.22	-59.53 ± 6.27	-37.61 ± 4.42
11	**1.5**	**0.5**	**-**	**0.5**	-34.65 ± 3.96	-59.03 ± 4.52	-30.45 ± 2.62
12	**1.5**	**2.0**	**-**	**0.5**	-40.13 ± 3.78	-38.76 ± 4.58	-37.52 ± 3.89
13	**1.5**	**-**	**0.5**	**-**	-36.11 ± 3.58	-56.12 ± 4.54	-43.97 ± 5.79
14	**1.5**	**-**	**2.0**	**-**	-51.78 ± 6.34	-55.99 ± 3.56	-46.87 ± 4.03
15	**1.5**	**-**	**0.5**	**0.5**	-37.74 ± 5.15	-40.16 ± 3.49	-34.50 ± 2.70
16	**1.5**	**-**	**2.0**	**0.5**	-40.31 ± 3.89	-41.45 ± 3.44	-40.88 ± 4.08

Values reported are the means and standard deviations (n = 20, 95 % confidence Interval, at 21.4 ± 0.5 ºC. Filled in gray are the better values, in the same group of polysaccharides (Tukey test $p<0.05$).

It was necessary to use Tween 80, once solutions of chitosan without Tween 80 present inferior values of *Ws* (results not show). The improvement of *Ws* with the addition of Tween 80 was also shown by Ribeiro, Vicente, Teixeira & Miranda (2007) and Choi, Park, Ahn, Lee & Lee (2002). Tween 80 acts reducing the superficial tension of the liquid and increasing the *Ws,* improving the compatibility of the solution and the cheese surface. The results obtained demonstrate that for chitosan the solutions with lower concentration of chitosan and without oil present better values of *Ws*. Samples 1, 2 and 6 do not present a statistically significant difference (Table 3).

To analyse statistically the values obtained for chitosan solutions, six subgroups (each subgroup corresponds to the values that do not have statistically significant difference between them), being subgroup six the one that maximizes the spreading coefficient.

Table 3 – Tukey test made to the spreading coefficient for the coatings of chitosan (C) in cheese (95 % of confidence).

Chitosan solution	Subgroup to α = 0,05					
	1	2	3	4	5	6
C.14	-51.78					
C.16		-40.31				
C.12		-40.13				
C.10		-38.95	-38.95			
C.9		-38.31	-38.31	-38.31		
C.15		-37.74	-37.74	-37.74		
C.7			-36.62	-36.62	-36.62	
C.8			-36.49	-36.49	-36.49	
C.13				-36.11	-36.11	
C.4				-35.76	-35.76	
C.11					-34.65	
C.3					-34.50	
C.5					-34.46	
C.6						-29.96
C.2						-29.81
C.1						-28.97
p – value	1.000	0.189	0.283	0.446	0.405	0.780

The samples that maximize the spreading coefficient for chitosan solutions were the samples 6, 2 and 1.

In the case of *G. triacanthos* the solutions with better *Ws* values were those containing oil. Samples 3, 12 and 15 (Table 4) do not present a statistically significant difference. The presence of oil in *G. triacanthos* coatings showed to decrease the *Ws.* The partly hydrophobic surface of the cheese presents a good adhesion to the solutions of *G. triacanthos* containing oil, eventually due to the ability of the solution with oil (more hydrophobic) to interact with cheese surface (Van Oss, 1995).

Table 4 – Tukey test made to the spreading coefficient of the coatings of *G. triacanthos* (GC) in cheese (95% of confidence).

***G. triacanthos* solution**	Subgroup to α = 0,05				
	1	2	3	4	5
GT.10	-59.53				
GT.11	-59.03				
GT.9	-58.97				
GT.2	-57.84				
GT.13	-56.12				
GT.14	-55.99				
GT.6	-54.79				
GT.7		-51.01			
GT.5		-49.69			
GT.1			-42.94		
GT.8			-41.93	-41.93	
GT.4			-41.69	-41.69	
GT.16			-41.45	-41.45	
GT.15			-40.16	-40.16	-40.16
GT.12				-38.76	-38.76
GT.3					-37.05
p – value	0.072	0.552	0.351	0.073	0.071

To analyse statistically the *Ws* values for *G. triacanthos*, five subgroups were created, being subgroup five the one that maximizes the spreading coefficient.

For the solutions made with *G. birdiae,* sample 11 was the better solution, presenting statistically significant differences from the other samples (Table 5). As in previous cases, the solutions containing oil present the best value of *Ws.*

The *Ws* values of *G. birdiae* are summarizes in Table 5, ten subgroups were created, being the subgroup ten the one that maximizes the spreading coefficient.

Table 5 – Tukey test made to the spreading coefficient for the coatings of *G. birdiae* (GB) in cheese (95% of confidence).

G. birdiae	**Subgroup to α = 0,05**									
solution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
GB.3	-55.46									
GB.7	-52.81									
GB.5		-49.62								
GB.8		-47.97								
GB.4		-47.37	-47.37							
GB.14			-46.87							
GB.1			-45.85							
GB.6				-45.69						
GB.13				-43.97	-43.97					
GB.16					-40.88	-40.88				
GB.9						-39.24	-39.24			
GB.10						-37.61	-37.61	-37.61		
GB.12							-37.52	-37.52		
GB.2								-36.49	-36.49	
GB.15									-34.50	
GB.11										-30.45
p – value	0.075	0.150	0.634	0.349	0.058	0.066	0.362	0.629	0.068	1.000

When there were no statistically significant differences between polysaccharide solutions, it has been assumed that both were equally good on terms of wettability and that their differentiation must be made based on other criteria (such as water vapour, O_2 and CO_2 permeability, colour and opacity).

Water vapor permeability (WVP)

The three best samples of chitosan in terms of wettability were subsequently analyzed for WVP. Figure 1 shows that the values of WVP change with the integration of sorbitol and a high concentration of glycerol. With the addition of sorbitol the WVP decreases, as shown by Garcia, Martino & Zaritzky (2000), Hernandez-Muñoz, López-Rubio, Del-Valle, Almenar, & Gavara (2004) and Mc Hugh & Krochta (1994). Table 6 shows that sample 6 is significantly different from the other samples, with a lower value of WVP. With the increase of glycerol concentration sample 1 to sample 2 there not exist a statistically significantly difference.

For *G. triacanthos* (GT) coatings samples 3, 12 and 15 were analyzed and Figure 1 shows the differences between the samples 3, 12 and 15. Samples 12 and 15 showed a lower value of WVP without a statistically significant difference. Sample 15 has a statistically significant difference with sample 3 (Table 7). An increase of concentration of GT corresponds to a decrease WVP, while the sample with sorbitol showed the lowest value of WVP. This can be explained by the larger size and lower hygroscopicity of the sorbitol compared to glycerol,

reducing the ability to affect hydrogen bonding between polysaccharide chains (Hong and Krochta, 2003).

Figure 1 shows also samples 2, 11, 15 of the solution with *G. birdiae.* The lower WVP values were registered for samples 11 and 15 which are not statistically different between each other, but have a statistically significant difference with sample 2. Increasing the concentration of *G. birdiae* led to lower values of WVP.

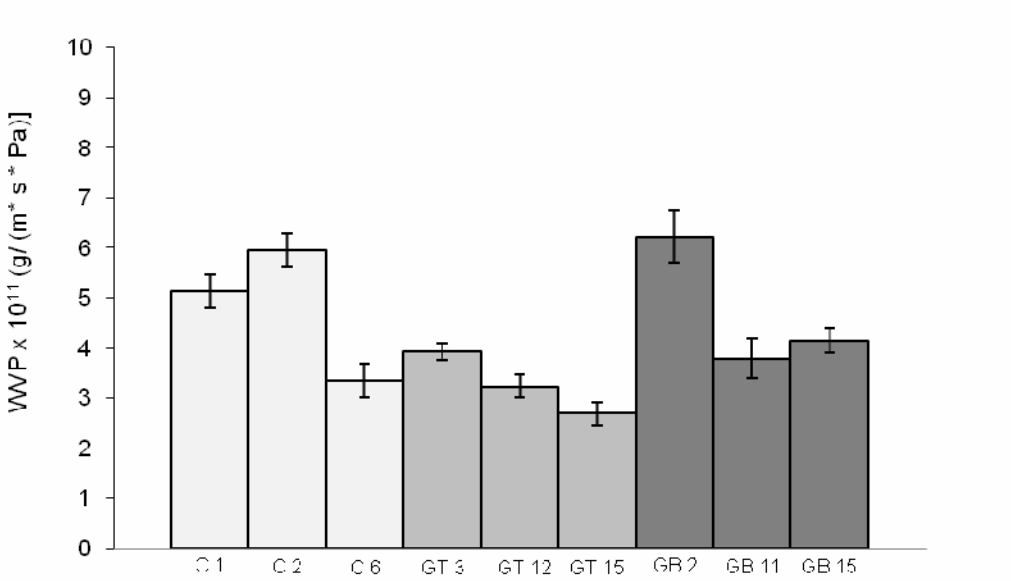

Figure 1 – Water vapour permeability of chitosan (C), *G. triacanthos* (GT) and *G. birdiae* (GB) samples (n = 3, 95 % confidence interval, at 22.1 ± 0.4 ºC).

The addition of oil promoted a decrease of WVP in *G triacanthos* and *G. birdiae* solutions. In this line, Hernandez (1994) indicated that WVP occurs through the hydrophilic portion of the film, depending of the hydrophilic-hydrophobic ratio of the films. Avena-Bustillos and Krochta (1993) showed that WVP decreases with the addition of beeswax to sodium caseinate films. Also Péroval et al. (2002) showed that arabinoxylan films with hydrogenated palm oil have lower WVP values than film without oil. Pranoto, Vilas, Salokhe & Rakshit (2005) showed similar results with alginate-based film containing garlic oil.

Oxygen permeability (O_2P)

Figure 2 presents the O_2P as measured for samples 1, 2 and 6 with chitosan. The samples with higher concentration of plasticizer have statistically higher values of O_2P (Table 7) than the samples with lower concentration. Similar results were also shown by Caner, Vergano, & Wiles (1998). The plasticizer decreases the intermolecular attractions between polymeric chains, facilitating the penetration of gas molecules (Kester & Fennema, 1989). With the addition of sorbitol the O_2P value decreased, as shows in sample 2 and sample 6. This difference can be explained by the different molecular size and hygroscopicity of sorbitol and glycerol.

The values of O_2P of the samples of *G. triacanthos* are presented in Figure 2. Sample 12 has the lower value (statistically different) of O_2P while corresponding to the higher concentration of plasticizer. In this case a higher concentration of the plasticizer decreases O_2P. Garcia, Martino, & Zaritzky (2000) found similar results for starch-based films. The addition of plasticizer decreases the presence of pores and cracks, improving the dispersion and decreasing the gas permeability (Garcia, Martino & Zaritzky, 2000).

There were no statistically significant differences for the solutions of *G. birdiae* in terms of O_2P (Table 7).

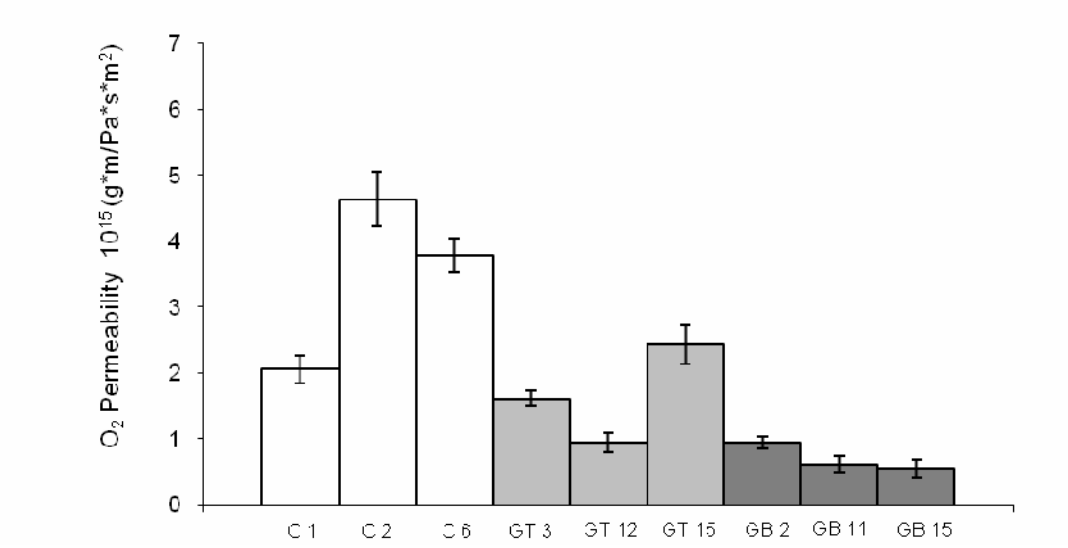

Figure 2 – O_2 permeability of chitosan (C), *G. triacanthos* (GT) and *G. birdiae* (GB) samples (n = 3, 95 % confidence interval, at 21.9 ± 0.3 ºC).

Carbon dioxide permeability (CO_2P)

Figure 3 shows the comparison of CO_2 permeability values for the different polysaccharides. The samples with a lower value of CO_2P for chitosan films were samples 2 and 6 and they not present a statistically significant difference between themselves. Sample 2 and 6, how ever, shows, a statistically significantly difference from sample 1 (Table 7). These results seem to indicate that samples with a higher concentration of plasticizer have a lower value of CO_2P.

For *G. triacanthos* the increasing of the polysaccharide concentration and the addition of sorbitol decrease the CO_2P. Sample 3 shows a statistically significant different from sample 12 and 15 (Table 6).

G. birdiae samples, display a very significant decrease with the increase of polysaccharide concentration. Also here, the addition of sorbitol decreases the value of CO_2P, as shown by Garcia, Martino & Zaritzky (2000).

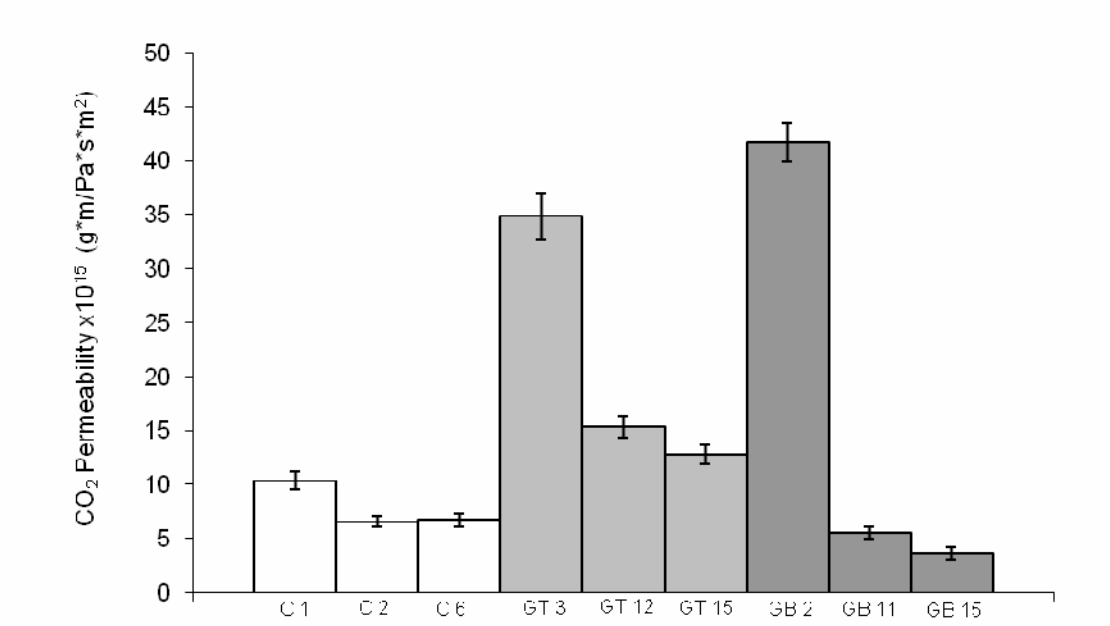

Figure 3 – CO_2 permeability of chitosan (C), *G. triacanthos* (GT) and *G. birdiae* (GB) samples (n = 3, 95 % confidence interval, at 21.7 ± 0.6 ºC).

Solubility, opacity and cromaticity

Table 6 presents the values of solubility, opacity and chromaticity *L** for all of the samples analyzed.

The solubility of the samples of chitosan was compared, and it was shown that the presence of sorbitol increases the solubility of chitosan films. The increase of the concentration of GT solutions decreases de solubility of the films. Further the results for the samples 12 and 15 demonstrate that the samples containing sorbitol display a higher solubility. The GB films do not present statistically significant differences between the analyzed samples. Comparing the three different polysaccharides it is possible conclude that GB is the polysaccharides with the lowest solubility and GT the one with the highest values.

The opacity means a smaller transparency, important to the light incidence in the cheese (Cuq, Gontard, Cuq & Guilber, 1996). Opacity values increase with the concentration in polysaccharide for samples of GT and GB, being the samples with sorbitol and oil those with a higher value of opacity. The addition of lipid caused the films to become whitish. Table 6 shows that the incorporation of corn oil in the films increased the opacity. Yang & Paulson (2000) demonstrated that also gellan film has increased opacity with the increase of lipid concentration.

The values of chromaticity *L** (Table 6) have different rages depending on the polyssacharide used. That was showen by Gennadios, Weller, Hanna & Fronning (1996) where the values for albumen films were between 95.67 and 96.20; for wheat protein films the reported values were between 83.30 and 89.70 (Rayas, Hernandez & Perry, 1997). The lower values in this work are presented by *G. birdiae* film.

Values of *L** decrease with the concentration of polysaccharide for samples of GT and GB, being the samples with sorbitol and oil those with a lower value.

Table 6 – Values of solubility, opacity and chromaticity *L** in the films

Solution		Solubility	Opacity	Chromaticity *L**
Chitosan	**1**	28.03 ± 1.31 ^{a}	4.41 ± 0.19 ^{a}	92.41± 1.38 ^{ab}
	2	27.14 ± 1.24 ^{a}	3.38 ± 0.19 ^{b}	93.77± 1.54 ^{a}
	6	64.86 ± 0.95 ^{b}	4.24 ± 0.33 ^{a}	94.34 ± 0.40 ^{a}
G. triacanthos	**3**	60.19 ± 2.04 ^{a}	5.62 ± 0.68 ^{a}	91.20 ± 0.83 ^{abc}
	12	42.38 ± 2.85 ^{b}	5.27 ± 0.15 ^{a}	85.53 ± 0.76 ^{d}
	15	52.43 ± 2.59 ^{c}	8.82 ± 0.40 ^{b}	86.41 ± 1.21 ^{def}
G. birdiae	**2**	23.70 ± 3.30 ^{a}	5.27 ± 0.49 ^{a}	90.01 ± 0.38 ^{be}
	11	22.56 ± 0.80 ^{a}	9.89 ± 0.61 ^{b}	84.97 ± 2.32 ^{dfg}
	15	22.24 ± 1.03 ^{a}	13.03 ± 0.29 ^{c}	88.20 ± 0.37 ^{cdfg}

Values reported are the means and standard deviations (n = 3, 95 % confidence interval). Different superscript letters indicate a statistically significant difference (Tukey test $p < 0.05$).

Criteria for choosing a coating

When choosing an adequate coating composition for the cheese under consideration, there are a number of criteria which should be met. Some of those (such as wettability) have already been considered. Others, such as gas transport properties and opacity, should be met in order to:

- Decrease the water loss in the cheese (i.e, lower WVP values);
- Decrease the O_2 permeability (i.e. lower O_2P values), once the oxygen in contact with the cheese contributes to the oxidation of fats and to the growth of undesirable microorganisms (Robertson, 1993);
- Increase the shelf-life of cheese, by increasing the lag-phase of growth of coliforms, yeasts, moulds and gram-negative spoilage bacteria (Mannheim & Soffer, 1996; Fedio*,* Macleod & Ozimek, 1994), i.e. high CO_2P values;
- Decrease the light incidence in the cheese (light promotes fat oxidation) (Robertson, 1993) i.e. high values of opacity.

Having these criteria in mind, it is possible to select the best values of the permeability for CO_2, O_2 and water vapor (see Table 7). In Table 7, the variables (WVP, O_2P and CO_2P) were placed by decreasing order of importance.

This being so, sample it was chosen 12 was chosen as the best option for coating cheese despite of its value CO_2P being not the highest among those determination at this stage. In fact, previous works have shown that there are advantages and disadvantages both for low and high CO_2P values (Papaioannou, Chouliara, Karatapanis, Kontominas & Savvaidis, 2007) justifying the choice for an intermediate one.

Table 7 – Values of Water, O_2 and CO_2 permeability in the films; the best sample is printed in bold face.

Solution		WVP (x10 $^{-11}$ g/m*s*Pa)	O_2P (x10 $^{-15}$ g/m*s*Pa)	CO_2P (x10 $^{-15}$ g/m*s*Pa)
Chitosan	1	5.13 ± 0.34 ^{a}	2.02 ± 0.28 ^{a}	10.42 ± 0.82 ^{a}
	2	5.61 ± 0.36 $^{a\,b}$	4.77 ± 0.79 ^{b}	6,61 ± 0.50 ^{b}
	6	3.07 ± 0.34 $^{c\,d}$	3.74 ± 0.34 ^{c}	6.76 ± 0.72 ^{b}
G. triacanthos	3	3.93 ± 0.17 c	1.63 ± 0.16 ^{a}	34.88 ± 2.17 ^{c}
	12	**3.24 ± 0.23 $^{e\,c}$**	**0.86 ± 0.06 ^{d}**	**15.35 ± 0.99 ^{d}**
	15	2.69 ± 0.23 $^{e\,d}$	2.42 ± 0.47 ^{a}	12.84 ± 0.91 $^{d\,a}$
G. birdiae	2	6.21 ± 0.52 b	0.95 ± 0.08 ^{d}	41.71 ± 1.80 ^{e}
	11	3.79 ± 0.40 c	0.61 ± 0.13 ^{d}	5.55 ± 0.53 ^{b}
	15	4.14 ± 0.24 c	0.55 ± 0.14 ^{d}	3.66 ± 0.54 ^{b}

Values reported are the means and standard deviations (n = 3, 95 % confidence interval). Different superscript letters indicate statistically significant difference (Tukey test $p < 0.05$). Filled in gray are the better values.

O_2 and CO_2 transfer rates in cheese

Cheese was coated using a solution with the formulation of sample 12 of *G. triacanthos*, and its O_2 and CO_2 transfer rates were compared with those of cheese without coating.

The gases were measured during 45 hours and the gas transfer rate was calculated and the results are presented in Figure 4. The coated cheese permits lower gas exchange in cheese. Figure 4 also shows that the rate of CO_2 production is higher than that of O_2 consumption.

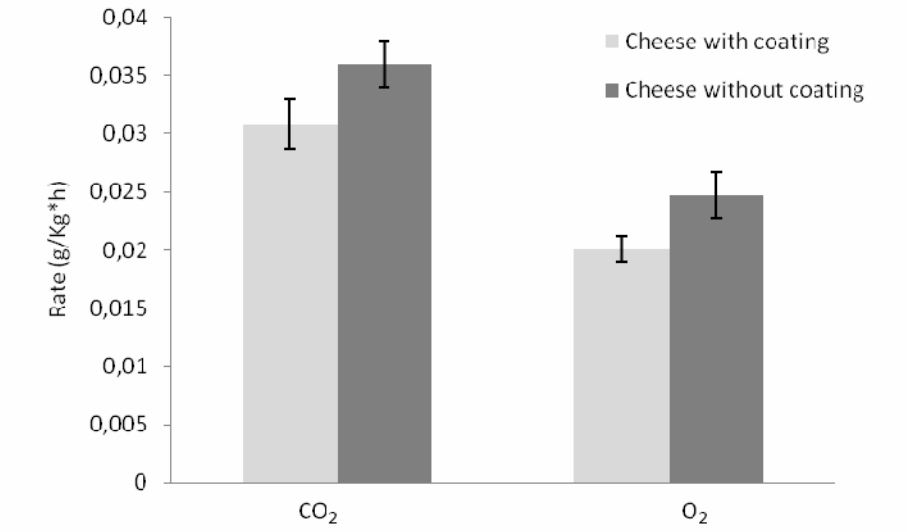

Figure 4 – O_2 and CO_2 transfer rates in cheese at 21.86 ± 0.76 ºC (n = 2, 95 % confidence interval).

Weight loss and relative humidity

The coated cheese presents a relative weight loss of 0.11 ± 0.04 %, while the cheese without coating loses 0.84 ± 0.07 %. Therefore, the coating allows a very significant decrease in the weight loss (ca. 13x of the value in the absence of coating).

The relative humidity, inside the jar, increase rapidly and at the end of the experiment it reached 100 %.

After 45 hours the cheese began to show fungal decay, most by occurring the uncoated cheese. This is very clear from Figure 5.

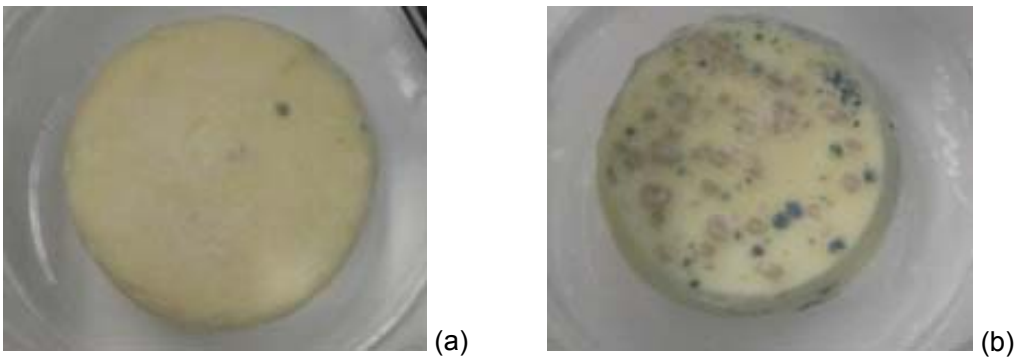

Figure 5 – Cheese in jar, with coating (a) and without coating (b). Note the fungal growth at the surface of the cheese in b).

Conclusions

This work presents a comparative analysis of polysaccharides from different novel species used in different formulations when used as edible coatings for cheese.

The cheese has surface and critical tension values of 37.79 mN/m and 18.33 mN/m respectively. It presents a surface of low energy and therefore the Zisman method was used to determine coatings' wettability as represented by the spreading coefficient (*Ws*).

The best values of the *Ws* were obtained with chitosan for the samples with lower values of chitosan concentration. When using *G. triacanthos* the solutions with better *Ws* were those containing oil (samples 3, 12 and 15). In the case of the solutions of *G. birdiae* sample 11 (1.5 % polysaccharide, 0.5 % glycerol and 0.5 % oil) was the best coating. The three best coatings of each polysaccharide in terms of *Ws* were further evaluated for gas permeability (water vapour – WVP – oxygen - O_2P – and carbon dioxide - CO_2P), colour and solubility.

In chitosan coatings the WVP decreased with the addition of sorbitol, increasing with a high concentration of glycerol. For *G. triacanthos* and *G. birdiae* the lower WVP values were found for samples with higher concentrations of polysaccharide.

The films of chitosan with higher concentration of plasticizer have values of O_2P statistically higher than those of the samples with lower concentration. Sample 12 (1.5 % polysaccharide, 2.0 % glycerol and 0.5 % oil) of *G. triacanthos* has the lower value of O_2P having a higher concentration of plasticizer when compared with the other samples. For the solutions of *G. birdie* the increase of polysaccharide concentration decreases O_2P.

In chitosan coatings, samples with a higher concentration of plasticizer have a lower value of CO_2P. For *G. triacanthos* the increase of the polysaccharide concentration and the addition of sorbitol decrease the value of CO_2P. For *G. birdiae* samples, there is a significant decrease of CO_2P with the increase of GB concentration and with the addition of sorbitol.

The opacity, solubility and *L** values for the tested films change in the presence of sorbitol and with the increase of polysaccharide concentration.

The better solution to coat the cheese was chosen by the lower value of WVP (related with the decrease of water loss) and the lower value of O_2P, corresponding to the sample 12 of *G. triacanthos*.

This solution was used to coat the cheese and the gases transfer rates were measured. The cheese with coating generally presents lower gas transfer rates (in general corresponding to 11 % of the values without coating), therefore contributing to decrease the relative weight loss of the cheese.

The present work can serve as guide for the use of new coatings for cheese, as an alternative to the synthetic coatings, and may also be a guide for the study of future novel materials for this purpose.

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